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6. AUTHORS Scott R Crittenden	5d. PROJECT NUMBER
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Approved for Public Release; Distribution Unlimited

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14. ABSTRACT

We have developed the ability to grow Shewanella putrifaciens and induce the production of flagella and, intermittently, pili. We have constructed partially conductive samples to enable KFM measurements of the potential of nanoscale wire-like objects and, finally, we have developed and published in the peer reviewd literature, a new measurement technique to determine the dielectric constant of thin layers in a non-contact, low force manner suitable for use on biological materials.

15. SUBJECT TERMS

AFM, SAM, electrogenic bacteria, nanowires, dielectric constant

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Scott Crittenden
UU	UU	UU	UU		19b. TELEPHONE NUMBER 803-777-7607

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Prescribed by ANSI Std. Z39.18

Report Title

FPR: Are the pili produced by electrogenic bacteria bionanowires?

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Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
FIona Oxsher	0.20	
Frank Grimmer	0.32	
Joe Bonvallet	0.09	
Jason Giamberardino	0.11	
FTE Equivalent:	**0.72**	
Total Number:	**4**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Bharat Kumar	0.33
FTE Equivalent:	**0.33**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Scott Crittenden	0.07	
FTE Equivalent:	**0.07**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Chris Pasco	0.05	
FTE Equivalent:	**0.05**	
Total Number:	**1**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:......	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:......	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See attachement.

Technology Transfer

AMSRD-ARL-RO-SI Proposal Number: 54729-EV
Agreement Number: W911NF-08-1-0387

Title: Are the pili produced by electrogenic bacteria bionanowires?
PI: Scott Crittenden

Statement of problem studied:

The title of the grant proposal is most succinct in making this statement: Are the pili produced by electrogenic bacteria bionanowires?

While this remained and remains the ultimate question, focus shifted during the work to questions that came to be seen to be necessary preliminaries. Specifically, how does one measure the conductivity of a nanoscale soft object?

Summary of results:

As discussed in the previous IPR, most progress occurred after July 30th 2010 with the arrival of Dr. Bharat Kumar. Over the entire performance period, we have developed the ability to grow Shewanella putrifaciens and induce the production of flagella and, intermittently, pili. We have constructed partially conductive samples to enable KFM measurements of the potential of nanoscale wire-like objects and, finally, we have developed a new measurement technique to determine the dielectric constant of thin layers in a non-contact, low force manner suitable for use on biological materials.

In particular we have managed to measure the electrical response of Shewanella MR-1 flagella (though not pili) lying partially on a gold electrode and partially on an insulating substrate using the AFM in Kelvin Force mode, as indicated in figure 1.

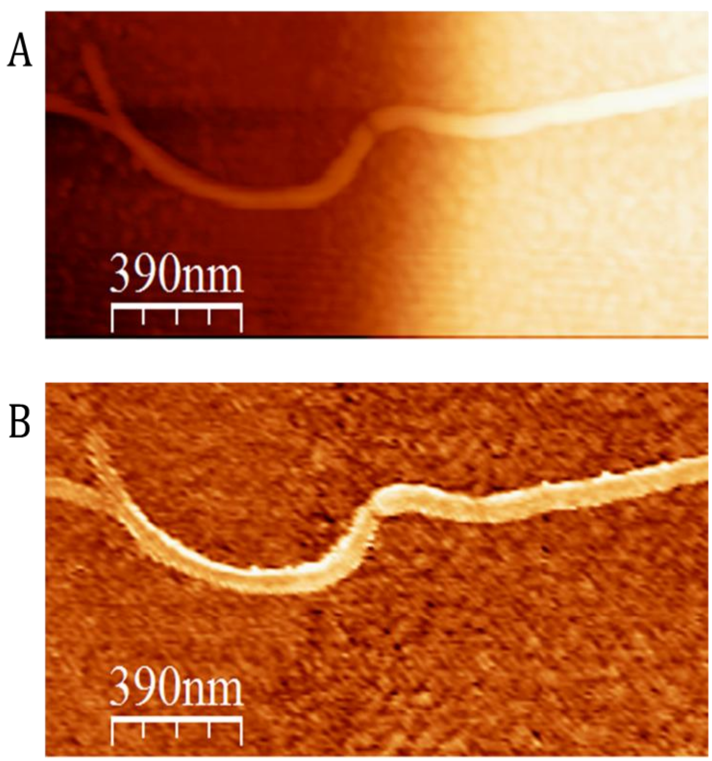

Figure 1: (a) Topography AFM image of flagellum. Color indicates height, dark being low and white high. High area to the right is the gold electrode.
(b) KFM derived image of potential difference between substrate and flagellum, showing a constant potential of the flagellum above both the baseline of the gold and

the baseline of the insulating mica.

Were the flagellum conductive, one would expect that any potential applied to the gold electrode would raise the potential of the flagellum as well. In this case the measured potential, via KFM, should be constant along the length of the flagellum. As figure 2 shows, the flagellar potential tracks that of the surrounding surface, being constant over the gold and changing linearly with distance over the insulating substrate. A constant potential difference of ~150-200mV exists between the flagellum and the surrounding sample, consistent with a standard work-function difference between the two.

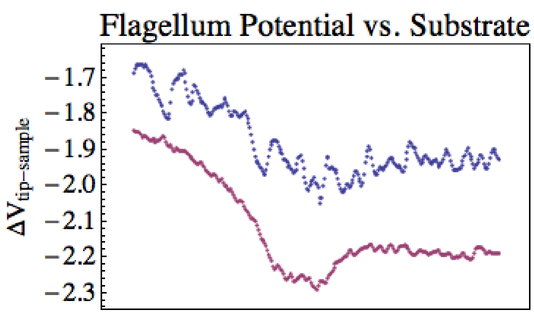

Figure 2: Potential along the flagellum in figure 1 (top blue data) compared to the surrounding surface potential (bottom red).

We have also seen pili (figure 3) though none of the electrical measurement data acquired was convincing.

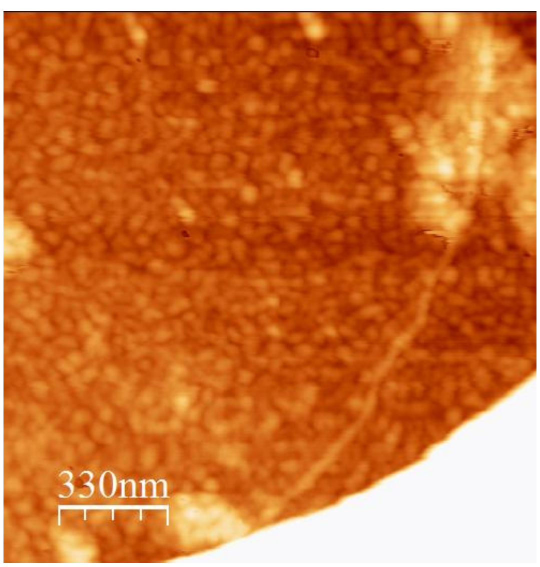

Figure 3: Topography image of pilus next to a bacterium on gold. Note that the length scale is approximately the same as for figure one, showing the much smaller diameter of the pilus compared to the flagellum.

The experience with the pili sent us in a different direction. We decided that there was no non-destructive and unambiguous way to determine the properties of the pilus-substrate electrical contact, thus rendering KFM measurements difficult to interpret. Either we don't know if the contact is good, or we can touch, and thereby crush with ~10GPa of pressure, part of the pilus, establishing a good contact but severely distorting the object under study, and therefore the meaning of the results.

We therefore sought, and eventually developed, a purely non-contact technique for measuring a relevant electrical property of delicate nanoscale objects. This was published in Nanotechnology[1] in December of 2011 (submitted October 5th, six weeks past the official end date of the grant, hence the '0' recorded for Publications above).

In particular, we developed a method to obtain capacitive forces and dielectric constants of ultra-thin films on metallic substrates using multifrequency non-contact atomic force microscopy with amplitude feedback in air. Capacitive forces were measured via cantilever oscillations induced at the second bending mode and dielectric constants were calculated by fitting an analytic expression for the capacitance to the experimental data.

The technique is notable because the high Q-factor of the second bending mode of the cantilever increases the accuracy of the capacitive measurements while the low applied potentials minimize the likelihood of variation of the dielectric constants at high field strength and of damage from dielectric breakdown of air, and no low-resistance mechanical contact to the object being measured is required. Previous techniques require either physical contact with the sample, which implies applied forces on the order of 10 GPa, more than sufficient to crush biological structures, or applied voltages of ~10V. Since the cantilever tip approaches the sample to distances of less than a nanometer, this implies electric fields of around 10 GV/m. Since the potential is maintained by an active power supply, this can result in dielectric breakdown of the air and nanoscale sparking across the tip-sample gap. For inorganic metallic samples, this may well not be problematical, but for biological samples such effects raise the issue of unknown and uncontrolled sample modification, rendering the interpretation of results difficult. Our technique allows the use of potentials on the order of 0.1 V, a two order-of-magnitude decrease.

The published work reports on self-assembled monolayers of thiolated alkanes on gold where analytic expressions relating the dielectric constant of the thin film to the measured parameters are possible. For arbitrarily shaped nanostructures, no such direct interpretation is possible and numerical fitting must be used. Work has continued in this direction, as well as in the direction of measuring electrical properties in liquid environments, where the pili are conjectured to actually serve as nanowires.

An example of the measurement and its result can be seen in figure 4.

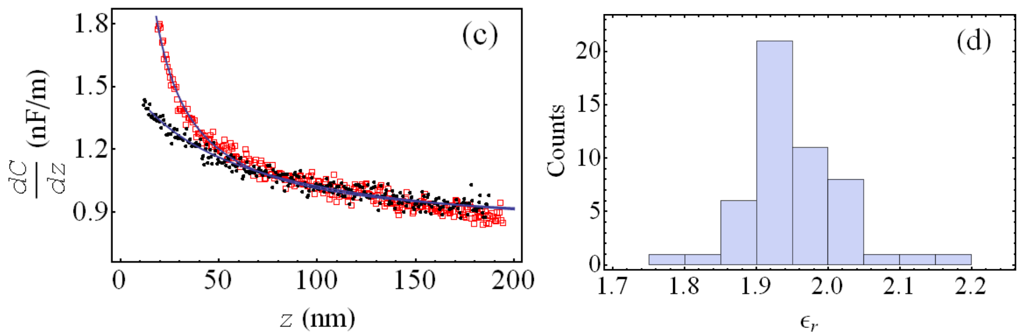

Figure 4: Figure labeled (c): Capacitive gradient (related to the dielectric constant, see [1]) over a carboxyl-terminated alkanethiol monolayer. Red squares are over the SAM, black dots are over bare gold—the measurement requires a comparison. Figure labeled (d): Deduced dielectric constant of the SAM for ~50 measurements like (c).

Besides the scientific accomplishments, a significant amount of training of scientific workers occurred. A group of three graduate students, each working part time on the grant, have gained significant experience in a variety of techniques from the use and accurate understanding of the atomic force microscope, to the growth of bacteria and the induction of particular biological responses (production of flagella and pili), to the preparation of single molecular layers on ultraflat substrates.

While I had hoped to definitively answer the question of the conductivity of the pili of electrogenic bacteria, I nevertheless believe that solid accomplishments were obtained despite not meeting that goal. We have developed a new and better technique for measuring electrical properties of soft nanoscale objects without damaging them and without the need to make good electrical and mechanical contact with the sample. This is a central problem in all nanowire measurements; how do you know you are measuring the intrinsic properties of the nanowire and not the contact? We have been able to sidestep that issue with out technique. Finally, the work has continued past the end of the grant and extended into measurements in liquid with another paper in preparation for submission before the end of the year. Finally, what we have learned during this effort has been published in the peer-reviewed literature and is being put to use in the service of another ARO funded research project.

Bibliography:

1 "Dielectric constants by multifrequency non-contact atomic force microscopy", B Kumar, J Bonvallet, S Crittenden, *Nanotechnology* **23** (2012) 025707